

# Alfalfa Leafcutting Bee Population Dynamics, Flower Availability, and Pollination Rates in Two Oregon Alfalfa Fields

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**ABSTRACT** Since the 1970s, it has become increasingly difficult for U.S. alfalfa seed producers to maintain *Megachile rotundata* (F.) populations used for alfalfa, *Medicago sativa* L., pollination. In 1998, we monitored *M. rotundata* population dynamics and foraging behavior, as well as alfalfa bloom and pollination rates in two fields in eastern Oregon. Despite marked differences in bee management, establishment was very similar in the two fields ( $\approx 0.5$  females per nesting cavity) and lagged peak bloom by  $\approx 2$  wk. Pollination rates increased from 0–10% in the first 3 wk to 80–90% in week 4–5. By then, *M. rotundata* females had difficulty finding untripped (nonpollinated) flowers and visited large numbers of already tripped or not fully matured flowers. *M. rotundata* progeny mortality was very high (54–78%). Estimated seed yields were similar in both fields. We contend similar seed yields, and improved bee production, could be accomplished with smaller bee populations, better timed with alfalfa bloom.

**KEY WORDS** *Megachile rotundata*, alfalfa pollination, foraging behavior, immature mortality, *Ascosphaera*

THE ALFALFA LEAFCUTTING BEE, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), is a cavity-nesting solitary bee native to Eurasia that was inadvertently introduced in North America in the 1930s (Stephen 1962). In the late 1950s, entomologists recognized the ability of the alfalfa leafcutting bee to pollinate alfalfa, *Medicago sativa* L., and shortly thereafter developed methods to manage alfalfa leafcutting bee populations (Bohart 1962, Stephen 1962, Hobbs 1967, Richards 1984). Currently, most alfalfa fields in southwestern Canada and the Pacific Northwest (Washington, Oregon, Idaho, Montana, and Nevada) are pollinated with alfalfa leafcutting bees, accounting for more than two-thirds of the alfalfa seed production in North America. In 1990, 10.9 million dollars was spent on alfalfa leafcutting bees in the United States (Peterson et al. 1992).

In the early stages of its establishment as a commercial pollinator, U.S. alfalfa seed producers were able to maintain or increase alfalfa leafcutting bee populations in alfalfa fields. Thus, the progeny obtained at the end of the flowering period could be used to pollinate alfalfa fields the following year, and alfalfa seed producers routinely sold surplus bees (Bohart 1972). However, starting in the mid 1970s, it has become increasingly difficult for U.S. alfalfa growers to maintain alfalfa leafcutting bee populations. Currently, population losses (difference between pa-

rental population released and live progeny recovered)  $>50\%$  are common in the Pacific Northwest (Peterson et al. 1992, Bitner 2000). For the past  $\approx 30$  yr, U.S. alfalfa seed producers have depended on the import of populations reared in Canada to maintain adequate pollination and commercial seed yields (Stephen and Fichter 1990, Peterson et al. 1992, Strickler 1996, Bitner 2000).

Chalkbrood, a disease caused by pathogenic fungi *Ascosphaera* spp. (Ascomycota: Ascosphaeraceae), has been invoked as the main factor determining the shift from routine bee population increases to chronic decreases in the United States (Peterson et al. 1992, Bitner 2000). Chalkbrood was first detected in Nevada alfalfa leafcutting bee populations in 1973 and rapidly spread to other U.S. alfalfa-growing areas, while remaining uncommon in Canada (Stephen et al. 1981). Notwithstanding the importance of chalkbrood, additional factors have probably contributed to the alfalfa leafcutting bee losses experienced in the past decades in the United States. Previous studies in the Pacific Northwest (Stephen 1981, Strickler 1996, Fauria 1998, Kemp and Bosch 1998, Strickler and Freitas 1999) identified several steps of the management process that may seriously hinder alfalfa leafcutting bee population growth: 1) Drifting (females that do not establish in the nesting materials provided) may significantly reduce the number of females establishing at the release sites; 2) Because large bee populations are concentrated in a relatively small nesting area, females spend long periods of time locating their nest; 3) As a result of the high bee densities used, floral resources become scarce, further limiting nesting progress and

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perhaps affecting provision quality; 4) Besides chalkbrood, high levels of "early immature mortality" (dead eggs and young predefecating larvae, sometimes referred to as "pollen balls" or unconsumed provisions) are prevalent in commercial alfalfa leafcutting bee populations; 5) Counts of live bees are made in the autumn; actual numbers of live females released per hectare are often lower than intended due to mortality during the winter and/or incubation.

Factors 1, 2, 3, and 5 above imply that alfalfa pollination in the United States is often accomplished with fewer alfalfa leafcutting bees, not working to their full pollinating capacities, than it is usually acknowledged. Interestingly, recommended alfalfa leafcutting bee densities for optimal alfalfa pollination have increased from 2,500–6,600 (Bohart 1962, Hobbs 1967, Gerber and Klostermeyer 1972) to 12,500 (Johansen et al. 1969) to the current 16,500–33,500 females per ha (Hobbs 1973, Richards 1984, Baird and Bitner 1991, Peterson et al. 1992). In practice, population densities as high as 40,000–50,000 females per ha are not uncommon in the Pacific Northwest (Baird and Bitner 1991, Peterson et al. 1992, Strickler 1996, Strickler and Freitas 1999).

In 1998, we monitored alfalfa leafcutting bee establishment and cell production, as well as flower availability and pollination rates throughout the blooming period, in two commercial alfalfa fields in eastern Oregon. At the end of the year, we assessed alfalfa leafcutting bee progeny production. Our objective was to assess the relative importance of factors (drifting, cell production, and progeny mortality) limiting alfalfa leafcutting bee population growth in the two fields and to identify appropriate changes to current management practices in the Pacific Northwest that would allow for better alfalfa leafcutting bee production while maintaining high seed yields.

### Methods and Materials

**Study Fields and Populations Released.** We worked in two commercial alfalfa fields near Nyssa, OR, that were representative of alfalfa leafcutting bee management in the Treasure Valley and routinely met or exceeded annual alfalfa seed production averages in the Pacific Northwest.

Field A measured 10.2 ha and was planted at a density of 33,267 plants per ha (estimate obtained from six 100-m<sup>2</sup> plots). Three modified cotton trailers (6 m in width by 3 m in height by 2 m in depth) were used as nesting shelters. Distance between shelters was  $\approx$ 180 m. We collected bee population dynamics data from two shelters. Each shelter contained 40 styrene boards (3540 nesting cavities per board). Nesting cavities were 9.5 cm in length and 6 mm in diameter. On 23 June, at 15.4% bloom, a first group of alfalfa leafcutting bee cells was released at a rate of  $\approx$ 140,000 cells per shelter. These bees were part of the alfalfa leafcutting bee progeny obtained by grower A the previous year and were managed as loose cells (cells punched out of the nesting cavities). Two weeks later, a second group of loose cells was released at a

rate of  $\approx$ 80,000 cells per shelter. These bees, also managed as loose cells, were purchased from Canada. To estimate percentage of live bees, we X-rayed  $\approx$ 950 and  $\approx$ 750 cells from the U.S. and Canadian populations, respectively, and counted cells with healthy-looking prepupae on the X-ray plates (Stephen and Undurraga 1976). These cells were then incubated at 22°C to record the sex of emerging bees. Hourly temperatures in the two shelters monitored were measured with data loggers.

Field B was located 21 km east of field A, measured 7.4 ha, and was planted at a density of 15,500 plants per ha (estimate obtained from six 100-m<sup>2</sup> plots). Nesting boards with cocooned adult bees were released on 24 June, at 16.4% bloom, in two large nesting shelters similar to those in field A. We collected bee population dynamics data from both shelters, which were separated by  $\approx$ 200 m. Each shelter had 71 wood boards (2,080 nesting cavities per board). Nesting cavities were 6 cm in length and 5 mm in diameter. Bees of this field were managed with the phase-out system, that is, nesting boards containing nests were set adjacent to empty boards in the shelters. Most of the bees released were the progeny obtained by grower B the previous year. Other bees were in styrene boards purchased from Canada. To estimate the number of cells released in field B, we cut one-row-thick slices from both types of nesting materials in the two nesting shelters. In early June, we X-rayed these slices and counted the number of cells in 75 Styrofoam and 190 wood-nesting cavities per shelter. The estimated number of cells released for emergence was 866,221 in shelter one and 1,309,065 in shelter 2. To estimate the number of live bees released and their sex ratio, we counted healthy-looking prepupae on the X-ray plates and sexed bees emerging from  $\approx$ 950 and  $\approx$ 700 cells from the U.S. and Canadian populations, respectively. Temperatures inside the shelters were measured as in field A.

**Alfalfa Leafcutting Bee Establishment and Nesting.** After alfalfa leafcutting bee emergence in the two fields, we conducted weekly night counts to estimate the numbers of females nesting at each of the two selected shelters per field. We used cardboard templates (cardboard frames delimiting an area including 15 by 15 [=225] nesting cavities) placed randomly on 10 nesting boards at each shelter. With the aid of a flashlight, we counted the number of females in each of the 225 nesting cavities within each template, as well as the number of plugged nests. At field B, we also sampled three phase-out boards (those containing release nests) per shelter. Thus, we surveyed 2,250–2,950 nesting cavities per shelter every week.

**Alfalfa Leafcutting Bee Progeny.** At the end of the flowering period, we removed one board per shelter and cut it in one-row-thick slices. In September, we X-rayed these slices and analyzed 1,500–2,000 cells per shelter. We only used one board per shelter to minimize damage to the grower's nesting materials and because all boards were thoroughly filled in all shelters (see below). We counted cells per nest, and assigned each cell to one of the following categories: live pre-

Table 1. Establishment of *M. rotundata* populations in two nesting shelters (1 and 2) in each of two alfalfa fields (A and B) in Nyssa, OR

Field	Shelter	Nesting cavities	Cells released	% live bees	Sex ratio ( $\delta/\varphi$ )	Live $\varphi$	$\varphi$ established	% $\varphi$ establishment	$\varphi$ established/nesting cavity
A	1	141,600	220,000	52.6	1.04	56,971	73,884	129.7	0.53
	2	141,600	220,000	54.9	0.86	65,045	77,534	119.2	0.56
B	1	147,680	866,221	34.5	1.92	102,169	67,554	66.1	0.45
	2	147,680	1,309,065	26.4	1.89	119,565	75,834	63.4	0.50

pupae, dead early immatures (eggs or young larvae), dead late immatures, chalkbrood, parasites (parasitoids, cleptoparasites and predators), second generation bees (empty cells from which bees had emerged as second generation adults). We combined these data with data on the total number of nests produced per board to estimate total progeny and total live progeny produced at each shelter.

A sample of cells with unconsumed provisions (early immature mortality) were dissected and examined under a stereomicroscope. Each cell examined ( $n$  of  $\approx 80$ –180 per shelter) was assigned to one of the following categories: no egg (provision with no trace of egg scar, indicating no egg was laid), missing egg (provision with egg scar but no bee egg or larva present, indicating the immature was either removed or fell off the uncapped cell), dead egg (provision with a dead egg), or dead young larva (provision with a dead predefecating larva).

To estimate individual offspring body size we used X-radiographs to measure the length and the width (precision 0.5 mm) of the first (innermost) and last (outermost) cocoons of 25 nests per nesting shelter. We assumed the first cocoon contained a female and the last cocoon a male (Stephen and Osgood 1965). We used cocoon length  $\times$  width to estimate prepupal weight (in milligrams) (prepupal weight =  $1.42$  [cocoon length  $\times$  cocoon width]  $- 10$ ,  $r^2 = 0.67$ ,  $P < 0.0001$ ,  $n = 142$ ).

**Flower Standing Crop and Pollination Rates.** To estimate flower standing crop (open flowers available), we conducted weekly flower counts at 10, 40, and 80 m away from each shelter. At each distance, we counted, at 0930–1100 hours, the number of nonpollinated flowers in three randomly chosen tagged plants. Pollinated alfalfa flowers are easily recognizable because their staminal column has been tripped (Free 1993). On each tagged plant, all racemes showing petals (blue-purple), and thus containing mature flowers and/or large buds, were counted. Then, a sample of 20–25 racemes per plant was closely examined to count the number of untripped flowers. We combined raceme counts and untripped flower counts to obtain total flowers open. To estimate pollination rates (percentage of tripped flowers), we examined samples of 20–25 racemes again in the evening (1900–2100 hours) and recorded tripped and untripped flowers. For week 5, 6, and 7 (when pollination rates were highest), we tested, separately for each field, for the effect of shelter and distance by fitting a two-way factorial without interaction to proportion data by using a binomial distribution and logit link (PROC GENMOD, SAS Institute 1999).

**Alfalfa Leafcutting Bee Foraging Activity and Behavior.** Once a week, at  $\approx 1100$  hours, we observed tagged plants for 10 min, and recorded the number of male alfalfa leafcutting bees, female alfalfa leafcutting bees, and other insects visiting their flowers. Previous observations in fields with high bee densities indicated that bloom availability declined from morning to afternoon. For this reason, on 23 July (week 5) we studied the behavior of foraging alfalfa leafcutting bees females in field B. In the morning (0900–1100 hours), midday (1200–1400 hours) and afternoon (1600–1800 hours), we recorded whether the flowers visited were fully developed (open) or not (i.e., the flag petal was not fully expanded and upright), as well as tripped or untripped ( $n$  of  $\approx 100$ –200 visits per time period). The ratio of open-untripped flowers versus other types of flowers visited at different times of the day was analyzed with the  $\chi^2$  test. We measured pollinating effectiveness by recording whether visits to untripped flowers resulted in the staminal column being tripped or not. We used 0.25- $\mu$ l capillary pipettes to measure nectar volumes in opened and unopened flowers, both tripped and untripped ( $n$  of  $\approx 30$  per flower type). Previous observations indicated also a decline in pollen-nectar collection from morning to afternoon. Thus, we observed alfalfa leafcutting bee females returning to the nesting boards in the morning, midday, and afternoon, and we recorded whether they were carrying a leaf piece or pollen ( $n$  of  $\approx 70$ –100 bees per time period). We tested for the effect of time of day and shelter by fitting a two-way factorial without interaction to proportion data by using a binomial distribution and logit link (PROC GENMOD, SAS Institute 1999).

**Seed Yields.** At harvest time (early September), we collected and counted all pods from the three plants surveyed at each distance-shelter-field. From a subsample of 50 pods per plant, we counted the number of seeds per pod and measured seed weight per pod.

## Results

**Release and Establishment.** Percentage of live individuals in the populations released was very low (field A: 51.8% [U.S. population] and 57.2% [Canadian population]; field B: 18.7% [U.S. population] and 87.6% [Canadian population]). Sex ratios (males/females) of emerged bees were lower in field A (U.S. population, 0.89; Canadian population, 1.03) than in field B (U.S. population, 2.30; Canadian population, 1.56). Table 1 summarizes establishment results at each shelter. Almost twice as many live females were released in field B as in field A, but the estimated

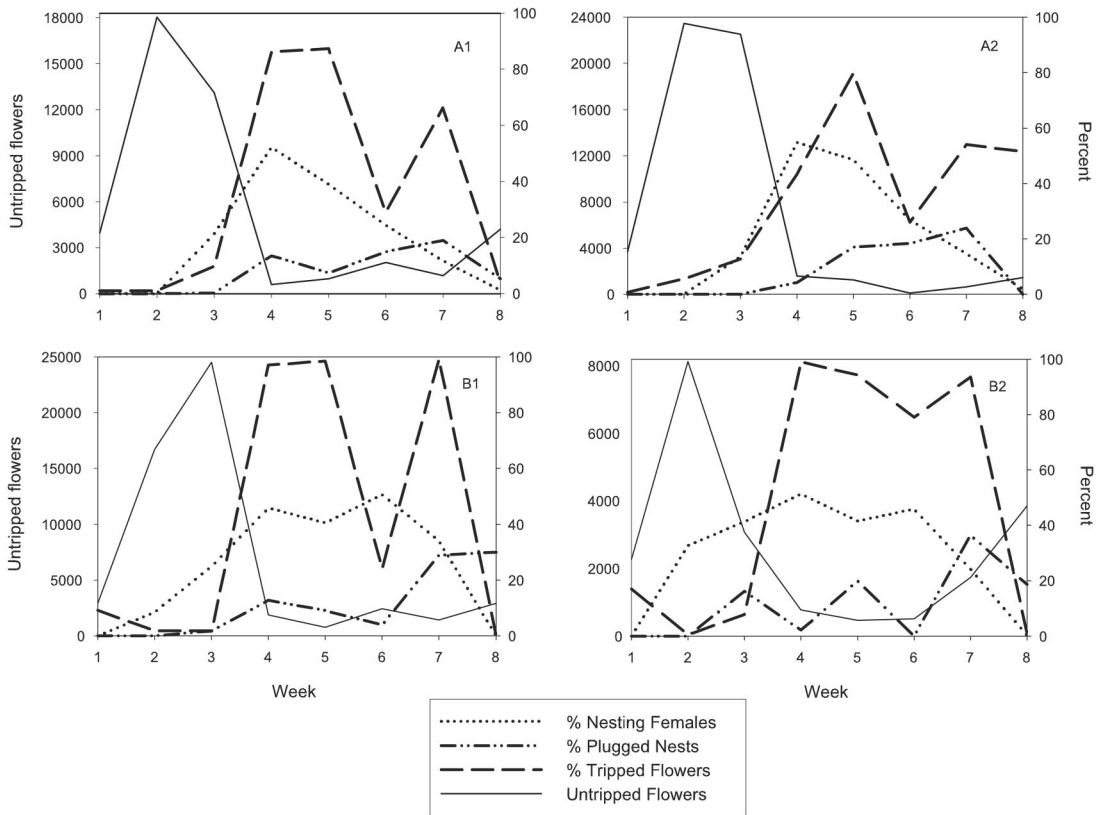


Fig. 1. Flower standing crop (untripped flowers), percentage of pollinated flowers (tripped flowers), percentage of cavities with *M. rotundata* (nesting females), and percentage of cavities with newly plugged nests (plugged nests) in shelters 1 and 2 of alfalfa fields A and B throughout the flowering period.

number of females established was remarkably similar across fields and shelters ( $\approx 70,000$  females per shelter). Female establishment (percentage of females released that established) was  $\approx 65\%$  in field B and exceeded  $100\%$  in field A. These results indicate that a large proportion of alfalfa leafcutting bee females do not nest at their emergence site, but rather drift between shelters (other alfalfa fields were common in the vicinity of fields A and B), and establish based on available nesting cavities. In both fields, females established at a rate of  $\approx 0.5$  female per nesting cavity (Table 1).

**Flower Standing Crop, Pollination Rates, and Nesting.** Bloom peaked in week 2–3 (1–2 wk after alfalfa leafcutting bee populations were released) in both fields (Fig. 1, untripped flowers). Pollination rates (Fig. 1, tripped flowers) were  $<10\%$  for the first 3 wk and then abruptly rose to  $80\text{--}100\%$  levels simultaneous with peak female establishment (Fig. 1, nesting females). Pollination rates remained high for the remainder of the flowering period, except for week 6, when rainy weather hindered alfalfa leafcutting bee activity. Nest production was very low for the first 3 wk (Fig. 1, plugged nests). Thus, most nests were completed during the second half of the blooming period, when flower availability was low.

More female alfalfa leafcutting bees per plant were recorded at 10 and 40 m from the nesting shelters than at 80 m (Fig. 2). However, tripping percentages were, for most of the blooming period, similar at 10, 40, and 80 m (Fig. 3). Both in weeks 5 and 7 (when tripping rates were highest; Fig. 1), tripping rates within a field did not vary between shelters (all  $\chi^2 < 2.58$ ;  $df = 1, 14$ ; all  $P > 0.1$ ) or among distances (all  $\chi^2 < 1.44$ ;  $df = 2, 14$ ; all  $P > 0.5$ ) (Fig. 3). In week 6, when weather was marginal for foraging activity, tripping rates varied across distances in field A ( $\chi^2 < 11.9$ ;  $df = 2, 14$ ;  $P > 0.003$ ) and between shelters in field B ( $\chi^2 < 18.9$ ;  $df = 1, 14$ ;  $P > 0.0001$ ) (Fig. 3).

**Foraging Behavior.** The ratio of pollen–nectar collecting versus leaf piece collecting decreased throughout the day ( $\chi^2 = 48.9$ ;  $df = 2, 2$ ;  $P < 0.0001$ ), but it did not vary between the two shelters monitored ( $\chi^2 = 1.7$ ;  $df = 1, 2$ ;  $P > 0.10$ ) (Table 2). Many of the flowers visited by alfalfa leafcutting bee females on week 5 were either already tripped or not fully developed, and the proportion of open untripped flowers visited decreased from morning to afternoon ( $\chi^2 = 72.9$ ;  $df = 2$ ;  $P < 0.0001$ ) (Table 3). Both immature (closed) and already tripped flowers contained nectar levels comparable to those in open untripped flowers (Table 4).

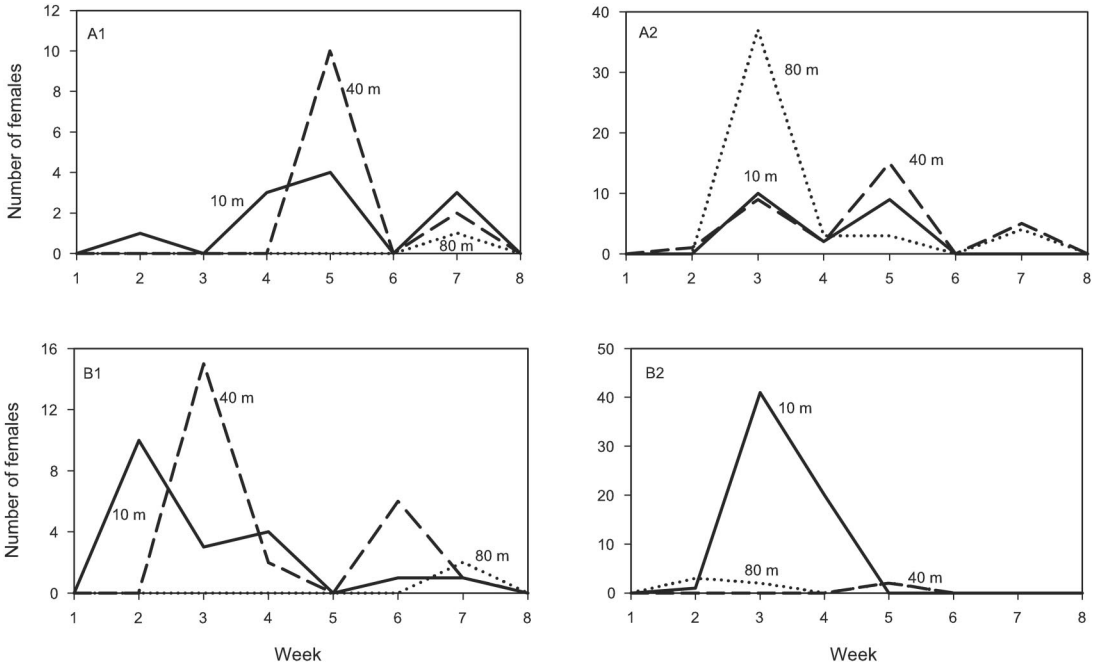


Fig. 2. Female *M. rotundata* frequency at alfalfa plants located 10, 40, and 80 m from nesting shelters 1 and 2 in fields A and B throughout the flowering period.

On unopened flowers, alfalfa leafcutting bee females forcibly pushed their proboscis in the corolla until they reached the nectaries, often tripping the flower in the process (72% tripping,  $n = 25$ ). Visits to tripped flowers (both open or unopened) did not result in

further pollination, because the stigma was already pressed against the standard. Tripping ability of alfalfa leafcutting bee males was poor (0%,  $n = 16$ ), but females pollinated 83.4% of the open, untripped flowers they visited ( $n = 157$ ).

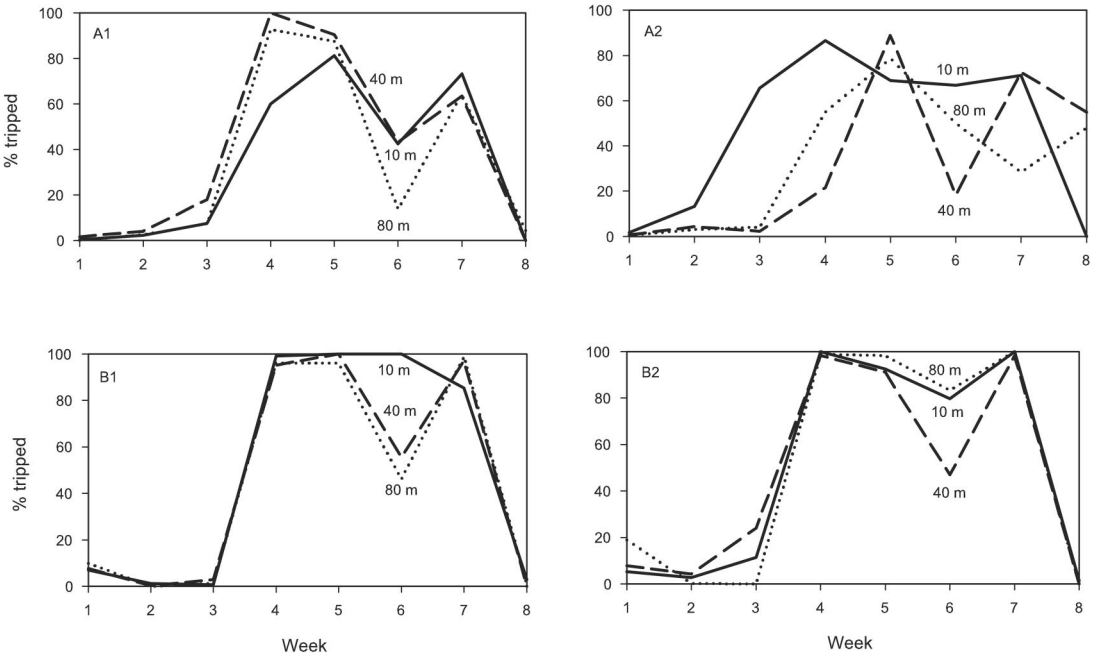


Fig. 3. Percentage of pollinated (tripped) flowers at 10, 40, and 80 m from nesting shelters 1 and 2 in alfalfa fields A and B throughout the flowering period.



Table 2. Ratio of *M. rotundata* females foraging for food (pollen–nectar) versus nesting material (leaf pieces) at three times of the day in two nesting shelters (1 and 2) in alfalfa field B in Nyssa, OR

Shelter	Time	n	Pollen/leaf
1	Morning	100	9.0
	Midday	100	1.3
	Afternoon	78	0.7
2	Morning	104	8.5
	Midday	103	1.5
	Afternoon	67	1.6

**Progeny.** Alfalfa leafcutting bee progeny produced by the end of the season (Table 5) was higher than the adult population released (Table 1) in field A but not in field B. Progeny mortality was lower in field A ( $\approx 45\%$ ) than in field B ( $\approx 78\%$ ) (Table 5). As a result, although more cells were produced in field B ( $\approx 750,000$  cells per shelter) than in field A ( $\approx 600,000$ ), production of live progeny in field A was almost twice that of field B (Table 5). The two fields had similar immature mortality (early and late immature mortalities combined), but chalkbrood incidence was much lower in field A (Table 5), where the loose cell system was used. Parasitism was low in both fields, as was the proportion of alfalfa leafcutting bees emerging to produce a second generation (Table 5).

Cells with unconsumed provisions (early immature mortality) were divided into several categories (Table 6). Dead young larvae (first to third instars) accounted for  $>50\%$  of early immature mortality in both fields, whereas dead eggs only accounted for  $\approx 10\%$ . In three of the four shelters sampled, as many as 20–30% of the unconsumed provisions analyzed had no scar on the surface, indicating no egg had been laid (Table 6). Approximately 7% of the unconsumed provisions had a scar on the surface, but no immature could be found, indicating the larva or egg had been removed, or the larva had crawled toward the entrance of the nest and fallen off while the cell and the nest were not capped.

An estimate of cell production per nesting female can be obtained by dividing the number of cells produced (Table 5) by the number of females established at each shelter (Table 1). This ratio was  $\approx 1.3$  times higher in field B ( $\approx 10$ –11 cells per female) than in

Table 3. Status (open or closed, tripped, or untripped) of flowers visited by *M. rotundata* males and females throughout the day in alfalfa field B in Nyssa, OR

Time	Flowers ( <i>n</i> )	Status of visited flowers			
		% open untripped	% open tripped	% closed untripped	% closed tripped
Females					
Morning	108	67.6	26.9	5.6	0
Midday	141	49.6	46.1	3.6	0.7
Afternoon	204	20.1	57.8	15.7	6.7
Males					
Morning	6	83.3	0	16.7	0
Midday	7	71.4	28.6	0	0
Afternoon	16	31.3	37.5	12.5	18.8

Table 4. Nectar volumes extracted from open untripped, open tripped, closed untripped, and closed tripped alfalfa flowers in field B in Nyssa, OR

Flower status	n	Nectar <sup>a</sup>	% flowers with measurable nectar
Open untripped	25	0.0194 $\pm$ 0.0082	32.0
Open tripped	36	0.0248 $\pm$ 0.0101	36.1
Closed untripped	28	0.0220 $\pm$ 0.0059	53.6
Closed tripped	28	0.0170 $\pm$ 0.0075	42.9

<sup>a</sup> Values are in microliters (mean  $\pm$  SE).

field A ( $\approx 7$ –9 cells per female) (Table 7). However, prepupal body size was  $\approx 1.2$  times higher in field A than in field B (Table 7). In field A, the population of live progeny recovered (631,995 bees) (Table 5) was 2.67 times greater than the live population released (236,434 bees) (Table 1). However, this increase is largely attributable to the drifting of females from adjacent fields (Table 1). In field B, the live population recovered (325,767 bees) was 0.51 times the live population released (644,530 bees).

**Seed Yields.** We obtained an estimate of seed yield by multiplying plant density in each field by mean seed weight produced by the nine plants sampled for each shelter. Despite differences in plant density between the two fields (A, 33,267 plants per ha; B, 15,500 plants per ha), seed yields thus estimated were remarkably similar across fields and shelters (A1, 2,084 kg/ha; A2, 2,377 kg/ha; B1, 2,334 kg/ha; B2, 2,041 kg/ha). These estimates were based on careful weighing of seeds produced by individual plants and thus undoubtedly overestimate commercial yields.

Discussion

**Timing of Alfalfa Leafcutting Bee Emergence.** Alfalfa flower standing crop was past its peak by the time alfalfa leafcutting bee females established in the two fields. This phenological mismatch is not uncommon in the Pacific Northwest. Strickler and Freitas (1999) reported a 2-wk lag between peak alfalfa bloom and alfalfa leafcutting bee nesting activity in seven fields in Idaho. Alfalfa leafcutting bee populations are routinely removed from wintering units in early June and incubated at 29–30°C for 3–4 wk (Richards and Whitfield 1988). Current management guidelines recommend that populations be placed in the shelters when male emergence is 75–100% complete and female emergence is 20–75% under way (Richards 1984, Murrell 1991). However, female emergence was barely starting when populations were placed in fields A and B in 1998. Mean hourly temperature in the nesting shelters between 25 June and 7 July (weeks 1 and 2 of this study) was 21.9°C in field A and 23.3°C in field B (range 3.7–36.7°C and 6.2–37.5°C, respectively). Female alfalfa leafcutting bees incubated at 22°C in the laboratory spent 8.4 d on average from adulthood to emergence compared with 3.3–4.3 d at 29–30°C (Kemp and Bosch 2000, 2001). After emergence and mating, females require two to three more days to begin nesting (Gerber and Klostermeyer 1972).

Table 5. *M. rotundata* cell production and progeny mortality in two nesting shelters (1 and 2) in each of two alfalfa fields (A and B) in Nyssa, OR

Field	Shelter	Nests produced	Cells produced	Live progeny n (%)	% early immature mortality	% late immature mortality	% chalkbrood	% parasitism	% second generation
A	1	117,245	646,020	350,401 (54.2)	23.0	13.3	3.2	1.4	4.9
	2	115,404	532,012	281,594 (52.9)	13.8	18.3	7.0	1.7	6.3
B	1	146,794	739,842	187,698 (25.4)	16.2	14.1	38.5	1.5	4.3
	2	146,942	747,935	138,069 (18.5)	18.8	15.4	44.1	1.3	2.0

**Alfalfa Leafcutting Bee Establishment and Cell Production.** Cell production per female was low (7–11 cells per female) compared with the 12–16 cells per female reported as usual cell productions in field populations (Gerber and Klostermeyer 1972, Baird et al. 1991, Peterson et al. 1992). Under favorable conditions, mean cell productions of 30–40 cells per female have been observed (Tirgari 1963, Gerber and Klostermeyer 1972; J.B., unpublished data). Cell production rates were well below the one cell per female per day obtained in other studies (Klostermeyer and Gerber 1969). Cell production per nesting female was  $\approx 1.3$  times higher in field B than in field A. However, mean offspring weight was  $\approx 1.2$  times higher in field A, making estimates of progeny weight produced per female similar in both fields. The smaller offspring size in field B may be explained by the smaller nesting cavity size in this field (Klostermeyer 1982). Female establishment per nesting cavity was very similar in the two fields studied. These results suggest that nesting resources (in addition to floral resources) may be limiting under the current alfalfa leafcutting bee densities used in alfalfa fields. Nesting boards were almost full in both fields ( $\approx 82$  and  $\approx 99\%$  cavities contained nests in fields A and B, respectively). Negative interactions among nesting females (fights and nest usurpation) and extended nest location times were prevalent in both fields (unpublished data).

**Progeny Mortality.** High levels of progeny mortality further reduced alfalfa leafcutting bee population growth. The mortality levels found in fields A and B are by no means uncommon in the Pacific Northwest (Strickler and Booth 1998). Early immature mortality has been attributed to several factors, including extreme temperatures (Tirgari 1963, Whitfield and Richards 1992, Richards 1996). It also has been attributed to nectar dearth (Tirgari 1963, Stephen and Fichter 1990, Strickler 1996, Kemp and Bosch 1998, Strickler and Freitas 1999). In another megachilid, *Osmia lig-*

*naria* Say, high levels of early immature mortality occurred when high population densities were managed in an apple–pear orchard, and larvae were unable to chew through the dry surface of provisions receiving insufficient nectar (Torchio 1985). However, a significant proportion ( $>30\%$ ) of alfalfa leafcutting bee cells from fields A and B with unconsumed provisions, contained no immature. Under the very high densities occurring at the nesting shelters (approximately two cavities per female), alfalfa leafcutting bee females have difficulty in finding their nest (Fauria 1998, Guédot et al. 2003, unpublished data), and some immatures are removed from their nests by other females (Gerber and Klostermeyer 1972) or accidentally fall from the nesting cavity entrance in nests that remain uncapped for long periods of time. We encountered alfalfa leafcutting bee larvae on the deck of all four nesting shelters.

Chalkbrood was high in field B but low in field A. High levels of spore deposition in the pollen–nectar provision were expected in field B, because chalkbrood incidence in the parental U.S. population was  $\approx 30\%$ , and populations were released within their natal nests (phase-out system), which forces bees to emerge through infested larval cadavers and thus become dusted with spores (Vandenberg et al. 1980). Additionally, emergence boards were left in the nesting shelters throughout the nesting period in field B, further facilitating chalkbrood spread. Chalkbrood levels were low in the parental populations of field A, and, probably because bees were managed as loose cells in this field, infection levels remained close to 5% in the progeny population. Chalkbrood was the mortality factor responsible for the dramatic differences in overall survival between the two fields.

In field A, live alfalfa leafcutting bee progeny recovered at the end of the blooming period was greater than the number of emerged adults placed in the field.

Table 6. Mortality in *M. rotundata* cells containing unconsumed provisions (early immature mortality) in two nesting shelters (1 and 2) in each of two alfalfa fields (A and B) in Nyssa, OR

Field	Shelter	n	% dead eggs	% dead larvae	% missing immature	% no egg scar
A	1	166	11.4	77.1	3.0	8.4
	2	84	7.1	56.0	7.1	29.8
B	1	146	11.0	55.5	10.3	23.3
	2	182	10.4	51.1	7.1	31.3

Table 7. *M. rotundata* cell production and prepupal body weight in two nesting shelters (1 and 2) in each of two alfalfa fields (A and B) in Nyssa, OR

Field	Shelter	Cell production <sup>a</sup>	♂ Prepupal wt <sup>b</sup>	♀ Prepupal wt <sup>b</sup>
A	1	8.74	46.77 $\pm$ 0.98	57.75 $\pm$ 1.22
	2	6.86	47.79 $\pm$ 0.86	54.81 $\pm$ 1.19
B	1	10.95	38.05 $\pm$ 0.54	41.89 $\pm$ 0.93
	2	9.86	40.54 $\pm$ 0.76	46.80 $\pm$ 0.89

<sup>a</sup> Cells produced/females established.

<sup>b</sup> Values are in milligrams (mean  $\pm$  SE).

However, because female establishment was  $\approx 124\%$  in this field, at least  $\approx 24\%$  of the cell production has to be attributed to females drifting from neighboring alfalfa fields. A Canadian study reported drifting levels of  $\approx 32\%$  or higher in several alfalfa fields (Goerzen et al. 1995). Our estimate of live progeny does not account for winter and spring (incubation) mortality. One of the populations released in field A was purchased assuming 4,500 live females per gallon (10,000 cells). These estimates are based on samples taken in the autumn, before wintering and incubation. According to our counts of healthy-looking prepupae, no  $> 3,000$  females per gallon emerged from this population. In a recent study, 16–22% of healthy-looking prepupae from commercial alfalfa leafcutting bee populations died during incubation (Strickler et al. 2000).

**Flower Standing Crop, Pollination Rates, and Seed Yields.** Large numbers of flowers were produced during the first 3 wk. Because alfalfa leafcutting bee females were not fully established by that time, tripping rates were low, and thus flower standing crop (and therefore pollen–nectar availability) remained high during this period. By week 4, two phenomena converged that caused flower standing crop to plummet. First, flowers pollinated during the previous week started to form pods (fully developed pods were noticeable in both fields in week 5). In alfalfa and other plants with indeterminate flower production, flower production decreases as energy and resources are diverted to fruit development (Stephenson 1981). Studies in Idaho (Strickler 1997, Strickler and Freitas 1999) recognized and modeled this relationship between pollination rates, pod development, and flower production, resulting in an exponential decrease in flower standing crop similar to our results in fields A and B. Second, with peak alfalfa leafcutting bee establishment, most new flowers produced were quickly pollinated, as indicated by the sudden increase in tripping rates (except in week 6 when weather was not conducive to alfalfa leafcutting bee activity).

As a consequence of this pattern of flower standing crop, most alfalfa leafcutting bee nesting took place at a time (second half of the flowering period) when pollen–nectar availability was low. Nectar production per flower is much higher early than late in the flowering period (Strickler and Freitas 1999). In fields A and B, flowers produced during the first half of the blooming period were visibly larger than flowers produced during the second half. Other results and observations provide further support that floral resources during the second half of the flowering period were scarce. Alfalfa leafcutting bee females had difficulty locating untripped flowers, and contrary to general belief, visited a large proportion of already tripped and not fully developed flowers. Alfalfa leafcutting bees obtained nectar, but not pollen, from these flowers. Females also characteristically switched from pollen–nectar foraging in the morning to leaf gathering in the afternoon. Finally, there were no differences in tripping rates in plants at increasing distances from the nesting shelters.

Potential seed yields as estimated in fields A and B were high. Average yields in the Pacific Northwest are 800–900 kg/ha (NASS 2001), and 2,200 kg/ha is considered an exceptionally high yield (Johansen 1991, Strickler 1996). Therefore, it could be argued that alfalfa leafcutting bee densities and the timing of nesting, although not optimal for bee production, were adequate for seed production. However, the mismatch between flower standing crop and pollination rates may have had consequences for seed production, too. Considering that not all alfalfa flowers need to be pollinated for maximum seed set (Pedersen et al. 1956), two scenarios are possible. First, pollination may have been insufficient during the first half of the blooming period. If so, energy that could have been invested in pod development was invested in the production of new flowers. Second, although flowers were tripped at a slow rate (unpollinated flowers last 3–5 d; Strickler and Freitas 1999), pollination may have been adequate during the first half of the blooming period. If so, pollination could have been accomplished, on younger flowers, with smaller bee populations whose nesting was better timed with peak bloom.

**Trade-Off between Seed and Bee Production?** At the current alfalfa leafcutting bee densities used for alfalfa pollination in the Pacific Northwest, alfalfa seed producers invest 20–25% of the annual value of their harvest sales in bees and pollination management (Peterson et al. 1992). Our results indicate that bee reproduction is limited first by insufficient floral resources during peak nesting and then by high mortality rates in the progeny produced. High bee densities are used because of the need to pollinate alfalfa quickly (Strickler 1996). This allows growers to avoid seed pest buildup and to harvest before the September rains (Stephen 1981, Mayer and Johansen 1991, Strickler 1997). Our results suggest that quick pollination also could be achieved with smaller bee populations, better timed with alfalfa bloom. It remains to be tested whether releasing alfalfa leafcutting bees earlier would negatively affect female establishment. Releasing bees too early might cause drifting by females unable to locate sufficient amounts of open flowers. This seems unlikely, because establishment progresses slowly at the beginning, and full female establishment does not occur until  $\approx 2$  wk after release. Alternatively, populations could be released with a larger proportion of bees emerged, instead of earlier. However, even if drifting increased, this might be compensated by several factors. First, using lower alfalfa leafcutting bee densities would actually decrease competition for floral resources, as well as nesting cavities. Second, females foraging on abundant floral resources would increase cell production rates and provision quality, resulting in improved progeny survival. Studies on two other Megachilidae have found a positive correlation between offspring size and survival (Tepedino and Torchio 1982, Bosch and Kemp 2004). Future studies should address timing between bee release and alfalfa bloom, as well as seed yields and bee population re-



turns in fields pollinated with smaller alfalfa leafcutting bee populations.

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